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(57) Abstract

The invention provides for a process for the production of a fermentable wort comprising the steps of: liquefying and saccharifying cereal material in the presence of one or more mashing enzymes, optionally subjecting the so–treated cereal material to filtration to obtain a fermentable wort, characterized in that during liquefaction and/or saccharification an enzyme having lipolytic activity is present. The cereal material may comprise crude cereal material, such as crude barley.

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APPLICATION OF LIPASE IN BREWING

Field of the invention

The present invention consists in making wort from unmalted cereals like barley with improved filterability. The invention also relates to the use of lipase in brewing in combination with routinely used mashing enzymes.

Background of the invention

Barley is the most important grain in use for making beer all over the world. Total lipid content in barley ranges from 3 to 5% of grains'dry matter. The distribution of lipids in barley is:

triglycerides	:	65-78%
glycolipids	:	7-13%
phospholipids	:	15-26%

Lipase is produced during germination of barley. The malt lipase thus obtained can hydrolyse most triglycerides into glycerol and free fatty acids. Consequently, barley grains contain higher amounts of lipids than malt grains. These lipids are able to build inclusion complexes with starch which makes starch degradation more difficult during mashing when barley is used as crude adjunct. Moreover lipids in the mash create an emulsion like medium leading to low filtrate rates. After wort filtration these lipids are found mainly in the spent grains causing rancidity, off-flavours, and others, which render cattlefeed application less acceptable. A minor amount (5-10%) is still found in the filtered wort with bad consequences on organoleptic properties of the final beer: no foam stability, staling, hazing.

Although much is known about the bad effects of lipids present when crude adjuncts are used in brewing, no solution has ever been proposed to

reduce these effects. Conversely much has been proposed to solve problems linked to the absence of endogenous enzymes when brewing with unmalted cereals.

Summary of the invention

The present invention enables to produce worts with high levels of crude adjuncts by combining the action of exogenous lipases with other mashing enzymes (e.g. amylase, protease, b-glucanase, endoxylanase). Also the invention enables to produce beer and any other alcoholic beverage, wherein a wort is obtained according to the invention. Also the invention includes the use of a lipase having 1,3-specificity on triglycerides, e.g. lipase from *Rhizopus oryzae*.

Accordingly, the invention comprises in one aspect the use of exogenous lipolytic activity in a process for making wort. The wort is preferably made from cereal material, more preferably crude cereal material. Preferred is crude barley.

According to another aspect of the invention, the lipolytic activity is a lipase or a phospholipase, preferably in conjunction with one or more mashing enzymes. The mashing enzyme may be one or more of the group consisting of: amylase, protease, beta-glucanase, (endo)xylanase and exopeptidase.

A preferred lipolytic activity has 1,3 specificity.

The lipolytic activity may be of fungal origin, preferably it originates from *Rhizopus oryzae*.

The invention provides for a process for the production of a fermentable wort comprising the steps of:

liquefying and saccharifying cereal material in the presence of one or more mashing enzymes, optionally subjecting the so-treated cereal material to filtration to obtain a fermentable wort, characterized in that during liquefaction and/or saccharification an enzyme having lipolytic activity is present.

The cereal material may comprise crude cereal material, such as crude barley.

The lipolytic activity may be a lipase or a phospholipase. Mashing enzymes if present are selected from one or more of the group consisting of: amylase, protease, beta-glucanase, (endo)xylanase and exopeptidase.

According to a further aspect of the invention a process is provided for making an alcoholic beverage, comprising the steps of making wort according to the invention and fermenting the said wort, optionally further treating the fermentate, to obtain the alcoholic beverage. A preferred alcoholic beverage is beer.

In another aspect the use is provided of an exogenous lipolytic enzyme for the treatment of cereal material.

Detailed Description of the Invention

The instant invention comprises a process of making wort and/or alcoholic beverages, characterised by the presence of lipase during one of the stages of its production.

The lipase is characterised as being exogenous, by which is meant that the lipase is not naturally present in the wort or alcoholic beverage making process. Wort is usually made from cereal material, which may accidentally have some lipase activity. If present at all, the lipase activity in cereal material is insufficient, as the Examples of the specification point out, to bring about the advantages of the invention. Therefore a lipase may be added from another source, such as a microbial or non-cereal vegetable source, which is one way of being exogenous, or from the same source as the cereal material from which the wort is made, but in higher amounts than usually present. Such higher amounts may be brought about by over-expressing genes coding for the lipolytic activity, or by adding a lipolytic activity of any source, including from a cereal source, which may be the same or different, at some stage in the process. Alternatively, the enzyme is already present prior to the onset of the process, such as will be the case when the lipolytic activity is present as a consequence of its production in the cereal, by

genetic modification of the cereal, or by adding it prior to the onset of the process.

The lipolytic activity may be a lipase or a phospholipase. A preferred lipolytic activity is one having 1,3-specificity (1 and 3 referring to the C-atoms in the alcohol backbone, usually glycerol, of the (phospho-)lipid, as these lipases have shown to have a very advantageous effect on filtration rates of the wort. Outstanding performance has been observed when lipolytic activity from *Rhizopus oryzae* or phospholipase C is used.

The cereal material may be any cereal material, although the advantages are most outspoken when at least part of the cereal material is "crude". With crude is meant non-malted. Those of skill in the art are aware of the fact, that malting is a complex and expensive process. In some countries, the import of malt is prohibited. To save money, or to circumvent any legislative hurdles, it is preferred to make beer, or any kind of alcoholic beverage which relies on malting for yield, taste and/or other organoleptic properties, the use of the process according to the invention is very advantageous. Not only the filtration rates are improved, also the properties of the beer, or any other alcoholic beverage is greatly improved by the use of lipases. Thus the invention will find use in making beer of crude cereals, or mixed brews (crude cereals mixed with malt). Preferred cereal material is barley, wheat, sorghum, oat or mixtures thereof.

Those of skill in the art know that in order to ferment cereal material, certain pretreatments are required in order to increase the content of glucose, or other fermentable sugars, freely available nitrogen. Whereas the malting process usually causes the content of fermentable nutrients to rise, with crude cereals, or mixed brews, use is made of exogenous enzymes. Liquefying and saccharifying enzymes, usually collectively referred to as mashing enzymes, comprise amylases (including alpha- and beta-amylases, hemicellulases (notably xylanolytic enzymes), (endo-)glucanases, proteases,

exopeptidases, and the like. It will be clear, that the use of lipase will advantageously be accompanied by the use of commonly used mashing enzymes. The conditions for liquefaction and saccharification are well known to those of skill in the art. Reference is made to handbooks of brewing.

The following examples illustrate the advantages of lipase in a process of making fermentable wort, and in making beer. However, the use of lipase in other alcoholic beverages, such as whisky and bourbon and other spirits is contemplated.

Examples

Experimental part

1-Lipase

Lipase activity is assayed by pH-stat monitoring the production of free fatty acids from olive oil. 1PLi unit is the amount of enzyme needed to produce 1μmol free acid per minute at pH 7.5 and 37°C for a neutral olive oil/water emulsion.

Lipase from *Rhizopus oryzae* is commercially available from Gist-brocades under the trademark Lipase 80000 having an activity of 80000 PLi/g.

2-other mashing enzymes

* liquefying amylase is from *Bacillus licheniformis*, commercially available from Gist-brocades under the trademark B.A.T.S.

* b-glucanase is from *Bacillus amyloliquefaciens*, commercially available from Gist-brocades under the trademark Filtrase L3000(+).

* protease is from *Bacillus amyloliquefaciens*, commercially available from Gist-brocades under the trademark Brewers' Protease (+).

* saccharifying amylase is from *Aspergillus oryzae*, commercially available from Gist-brocades under the trademark Brewers Fermex.

*) endoxylanase is from *Aspergillus niger*, commercially available from Gist-brocades under the trademark Lyxasan Forte.

Example 1

Wort was prepared from crude barley grains, variety PLAISANT.

Barley grains were ground with the EBC MIAG mill in order to make filter press type barley flour. 57g barley flour are added in 300ml water or aqueous solution of enzymes at 50°C. This temperature is maintained for 1 h ; it is then heated up to 63°C (1°C/min) and maintained at that temperature during 30 minutes. The medium is then heated up to 90°C

(1°C/min) and maintained at that temperature during 20 minutes. Water is added to compensate for water evaporation. The mash is then poured into a funnel containing Schleicher and Schull paper filter.

Mashing enzymes were applied in each brew at the following dosage (in g per T barley):

- B.A.T.S. : 300
- Filtrase L3000(+) : 17
- Brewers' protease (+) : 110
- Brewers Fermex : 2000

whereas Lipase 80000 and Lyxasan Forte were used at varied levels:

Assay	Lipase 80000	Lyxazan Forte	Volume (ml) filtered after 1h
	in g per T barley		
1	0	100	46
2	25	100	148
3	0	1400	82
4	25	1400	158
5	12	750	118

Results clearly show the influence of lipase on the filtration rate, which is stronger than the well known effect of endoxylanase.

Example 2

In this series wort was prepared from various malts using the same brewing diagram as in Example 1. However, mashing enzymes were different:

- assay 1 : no enzyme added
- assay 2 : 300g Filtrase Br (commercially available from Gist-brocades) per T malt
- assay 3 : 25g Lipase 80000 per T malt
- assay 4 : 300g Filtrase Br + 25g Lipase 80000 per T malt.

Malt from Korea

Assay	Volume (ml) filtered after 1h
1	45
2	63
3	53
4	53

Malt from China

Assay	Volume (ml) filtered after 1h
1	98
2	250
3	102
4	224

Results clearly indicate the poor effect of lipase in malt brews whereas a filtration enzyme (mix of amylase, protease and hemicellulases) usually applied show normal efficiency. Moreover no synergistic effect between lipase and filtration enzyme can be demonstrated.

Example 3

In this series we determine the optimal dosage of lipase to be applied. Wort was prepared from barley as in Example 1.

Mashing enzymes were the same as in Example 1. No endoxylanase was used in the series. Lipase addition was varied as shown below:

Assay	Lipase 80000 in g per T barley	Volume (ml) filtered after 45'
1	0	46
2	6.3	92
3	12.5	162
4	25	170
5	62.5	180
6	125	188
7	250	192
8	625	116
9	1,250	109

Results indicate that high performance is already obtained as from 12g/T whereas overdosing (>250g/T) has detrimental effect.

Example 4

In view of correlating lipase's specificity to brewing performance, we have compared different lipase and phospholipase commercially available.

Worts were prepared from barley as described in Example 1. Mashing enzymes, were the same as in Example 3. Lipolytic enzymes were varied as given below:

Assay	Enzyme	Commercial source	Dose for 1kg barley	Volume (ml) after 1h
1	none	-	0	46
2	Lipase 80000	Gist-broc.	2000 PLi	169
3	Novozyme 677	Novo Nordisk	2000 PLi	50
4	Phospholipase A2	Sigma	267 Sigma units	44
5	Phospholipase B	Sigma	133 Sigma units	42
6	Phospholipase C	Sigma	333 Sigma units	70
7	Phospholipase C	Sigma	1330 Sigma units	155
8	Phospholipase D	Sigma	1330 Sigma units	56

Lipase 80000 from *Rhizopus oryzae* has an absolute 1,3 specificity and preferably yields 1,2-diglycerides. Phospholipase C which yields 1,2-diglycerides too but from phospholipids, seems to perform nearly as well as Lipase 8000.

These results are rather surprising since malt lipase is given as non regiospecific lipase and malt phospholipase is given as being of B-type.

Claims

1. Use of exogenous lipolytic activity in a process for making wort.

2. Use according to claim 1, wherein the wort is made from cereal material.

3. Use according to claim 2, wherein the cereal material comprises crude cereal material.

4. Use according to claim 3, wherein the crude cereal material comprises crude barley.

5. Use according to any one of claim 1 to 4, wherein the lipolytic activity is a lipase or a phospholipase.

6. Use according to any one of claim 1 to 5, wherein in addition to the lipolytic activity one or more mashing enzymes are added.

7. Use according to claim 6, wherein the mashing enzyme is selected from one or more of the group consisting of: amylase, protease, beta-glucanase, (endo)xylanase and exopeptidase.

8. Use according to any one of claim 1 to 7, wherein the lipolytic activity has 1,3 specificity.

9. Use according to any one of claim 1 to 8, wherein the lipolytic activity is of fungal origin.

10. Use according to any one of claim 1 to 9, wherein the lipolytic activity originates from Rhizopus oryzae.

11. A process for the production of a fermentable wort comprising the steps of:
liquefying and saccharifying cereal material in the presence of one or more mashing and saccharifying enzymes, optionally subjecting the so-treated cereal material to filtration to obtain a fermentable wort, characterized in that during liquefaction and saccharification an enzyme having lipolytic activity is present.

12. A process according to claim 11, wherein the cereal material comprises crude cereal material.

13. A process according to claim 12, wherein the crude cereal material comprises crude barley.

14. A process according to any one of claim 11 to 13, wherein the lipolytic activity is a lipase or a phospholipase.

15. A process according to any one of claim 11 to 14, wherein in addition to the lipolytic activity one or more mashing enzymes are added.

16. A process according to claim 15, wherein the mashing enzyme are selected from one or more of the group consisting of: amylase, protease, beta-glucanase, (endo)xylanase and exopeptidase.

17. A process according to any one of claim 11 to 16, wherein the lipolytic activity has 1,3 specificity.

18. A process according to any one of claim 11 to 17, wherein the lipolytic activity is of fungal origin.

19. A process according to claim 18, wherein the lipolytic activity originates from Rhizopus oryzae.

20. A process making an alcoholic beverage, comprising the steps of making wort using a process according to any one of claims 11 to 19 and fermenting the said wort to obtain the alcoholic beverage.

21. A process according to claim 20, wherein the alcoholic beverage is beer.

22. Use of an exogenous lipolytic enzyme for the treatment of cereal material.

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/EP 99/02047

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12C5/02 C12G1/02 C12G3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12C C12G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	abstract	1-5

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

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